

## AMENDMENTS TO THE SPECIFICATION:

Please amend the paragraph beginning at page 31, line 12, as follows:

**FIG. 1A** presents the amino acid sequence about the site of fusion between CD4 (residues 1-369) and different receptor chains (SEQ ID NO:28, 29, 30, and 31). The underlined sequence shows the position of the amino acids encoded within the BamHI site used for fusion construction. The beginning of the transmembrane domain is marked with a vertical bar. The  $\eta$  sequence is identical to the  $\zeta$  sequence at the amino terminus, but diverges at the carboxyl terminus (Jin et al., Proc. Natl. Acad. Sci. USA 87:3319-3323 (1990)). **FIG. 1B** presents flow cytometric analysis of surface expression of CD4, CD4: $\zeta$ , CD4: $\gamma$  and CD4: $\eta$  in CV1 cells. Cells were infected with virus expressing CD4 chimeras or CD16<sub>PI</sub>, incubated for 9 hours at 37°C, and stained with phycoerythrin-conjugated anti-CD4 Mab Leu3A.

Please amend the paragraph beginning at page 34, line 3, as follows:

**FIG. 7A-B** shows characterization of the CD16: $\zeta$  chimeric receptor. **FIG. 7A** is a schematic diagram of the CD16: $\zeta$  fusion protein. The extracellular portion of the phosphatidylinositol-linked form of monomeric CD16 was joined to dimeric  $\zeta$  just external to the transmembrane domain. The protein sequence at the fusion junction is shown at the bottom (SEQ ID NO:32 and 33). **FIG. 7B** shows a flow cytometric analysis of calcium mobilization following crosslinking of the CD16: $\zeta$  chimera in either a TCR

positive or TCR negative cell line. The mean ratio of violet to blue fluorescence (a measure of relative calcium ion concentration) among cell populations treated with antibodies at time 0 is shown. Solid squares, the response of Jurkat cells to anti-CD3 MAb OKT3; solid triangles, the response of CD16:ζ to anti-CD16 MAb 3G8 crosslinking in the REX33A TCR<sup>-</sup> mutant; open squares, the response to CD16:ζ crosslinking in the Jurkat TCR<sup>-</sup> mutant line JRT3.T3.5; open triangles, the response to CD16:ζ crosslinking in Jurkat cells; crosses, the response to nonchimeric CD16 in Jurkat cells; and dots, the response to nonchimeric CD16 in the REX33A TCR<sup>-</sup> cell line.

Please amend the paragraph beginning at page 34, line 23, as follows:

**FIG. 8A-B** shows deletion analysis of cytolytic potential. **FIG. 8A** shows the locations of the ζ deletion endpoints. Here as elsewhere mutations in ζ are represented by the original residue-location-mutant residue convention, so that D66\*, for example, denotes replacement of Asp-66 by a termination codon (SEQ ID NO:34). **FIG. 8B** shows cytotoxicity assay results of undeleted CD16:ζ and salient ζ deletions. Hybridoma cells expressing surface antibody to CD16 were loaded with <sup>51</sup>Cr and incubated with increasing numbers of human cytolytic lymphocytes (CTL) infected with vaccinia recombinants expressing CD16:ζ chimeras. The percent of <sup>51</sup>Cr released is plotted as a function of the effector (CTL) to target (hybridoma) cell ratio (e/t). Solid circles, cytotoxicity mediated by cells expressing CD16:ζ (mfi 18.7); solid squares, cytotoxicity mediated by cells expressing

CD16:ζAsp66\* (mfi 940.2); open squares, cytolysis mediated by cells expressing CD16:ζGlu60\* (mfi 16.0); open circles, cytolysis mediated by cells expressing CD16:ζTyr51\* (mfi 17.4); solid triangles, cytolysis mediated by cells expressing CD16:ζPhe34\* (mfi 17.8); and open triangles, cytolysis mediated by cells expressing nonchimeric CD16 (mfi 591). Although in this experiment the expression of CD16:ζAsp66\* was not matched to that of the other fusion proteins, cytolysis by cells expressing CD16:ζ at equivalent levels in the same experiment gave results essentially identical to those shown by cells expressing CD16:ζAsp66.

Please amend the paragraph beginning at page 35, line 16, as follows:

**FIG. 9A-D** shows that elimination of the potential for transmembrane interactions reveals a short ζ segment capable of mediating cytolysis. **FIG. 9A** is a schematic diagram of the monomeric bipartite and tripartite chimeras. At the top is the CD16:ζ construct truncated at residue 65 and lacking transmembrane Cys and Asp residues. Below are the CD16:CD5:ζ and CD16:CD7:ζ constructs and related controls. The peptide sequences of the intracellular domains are shown below (SEQ ID NO:34, 36, and 37). **FIG. 9B** shows the cytolytic activity of monomeric chimera deletion mutants. The cytolytic activity of cells expressing CD16:ζ (solid circles; mfi 495) was compared to that of cells expressing CD16:ζAsp66\* (solid squares; mfi 527) or the mutants CD16:ζCys11Gly/Asp15Gly/Asp66\*, (open squares; mfi 338) and

CD16:ζCys11Gly/Asp15Gly/Glu60\* (filled triangles; mfi 259). **FIG. 9C** shows the cytolytic activity mediated by tripartite fusion proteins. Solid triangles, CD16:ζAsp66\*; open squares, CD16:5:ζ(48-65); solid squares CD16:7:ζ(48-65); open triangles, CD16:7:ζ(48-59); open circles, CD16:5; solid circles, CD16:7. **FIG. 9D** shows calcium mobilization by mutant and tripartite chimeras in the TCR negative Jurkat JRT3.T3.5 mutant cell line. Open circles, response of cells expressing dimeric CD16:ζAsp66\*; solid squares, response of cells expressing CD16:ζCys11Gly/Asp15Gly/Asp66\*; open squares, response of cells expressing CD16:ζCys11Gly/Asp15Gly/Glu60\*; solid triangles, response of cells expressing CD16:7:ζ(48-65); and open triangles, response of cells expressing CD16:ζ(48-59).

Please amend the paragraph beginning at page 37, line 6, as follows:

**FIG. 11A-B** shows alignment of internal repeats of ζ and comparison of their ability to support cytotoxicity. **FIG. 11A** is a schematic diagram of chimeras formed by dividing the ζ intracellular domain into thirds and appending them to the transmembrane domain of a CD16:7 chimera. The sequences of the intracellular domains are shown below, with shared residues boxed, and related residues denoted by asterisks (SEQ ID NO:38, 39, and 40). **FIG. 11B** shows the cytotoxic potency of the three ζ subdomains. Solid circles, cells expressing CD16:ζ (mfi 476); solid squares, CD16:7:ζ(33-65) (mfi 68); open squares, CD16:7:ζ(71-104) (mfi 114); and solid triangles, CD16:7:ζ(104-138)

(mfi 104).

Please amend the paragraph beginning at page 38, line 4, as follows:

**FIG. 15A-E** shows identification of residues in the FcR $\gamma$ II A tail which are important for cytolysis. **FIG. 15A** is a schematic diagram of the deletion constructs (SEQ ID NO:41). **FIGS. 15B** and **15C** shows calcium mobilization and cytolysis by carboxyl-terminal deletion variants of CD16:FcR $\gamma$ II A. **FIGS. 15D** and **15E** show calcium mobilization and cytolysis by tripartite chimeras bearing progressively less of the amino terminus of the intracellular tail of CD16:FcR $\gamma$ II A.

Please replace the current Sequence Listing with the Sequence Listing submitted with the concurrently filed Statement under 37 C.F.R. §§ 1.821-1.825.